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Nitrogen Control in Bacteria

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INTRODUCTION

Bacteria can utilize a wide range of nitrogen compounds as sole sources of cellular nitrogen. These range from simple inorganic compounds such as dinitrogen and nitrate to complex compounds including amino acids such as histidine and arginine or nucleosides such as cytidine. The assimilatory metabolic pathways of nitrogen metabolism can be divided into two classes: the pathways necessary for utilization of nitrogen from the extracellular medium and the biosynthetic pathways for intracellular production of nitrogen-containing compounds. The precise combination of pathways available depends on the organism in question, but in most cases the coordinated expression of the enzymes of nitrogen metabolism appears to respond primarily to the intracellular nitrogen pool. It is the genetic mechanisms underlying this control of bacterial nitrogen metabolism that form the subject of this review.

The foundations of our knowledge of nitrogen control are built almost entirely on research with enteric bacteria, notably *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella aerogenes*, and *Klebsiella pneumoniae*, and this early work was extensively reviewed by Tyler (244), Magasanik (159), Merrick (173), and Reitzer and Magasanik (208). However, there is now considerable evidence that many of the fundamental principles learned from these studies apply to the process of nitrogen control in other eubacterial genera. In the first part of this

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TABLE 1. GS enzymes in prokaryotes

Enzyme	Structural gene	Subunit mol wt	No. of subunits	Properties	K_m[a] (mM)	Covalent modification	Distribution
GSI	*glnA*	55,000	12	Heat stable	0.2	Adenylylation,[b] ADP-ribosylation[c]	Almost universal
GSII	*glnII*	36,000	8	Heat labile	0.2	Possibly[d]	*Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Streptomyces*, *Frankia* spp.
GSIII	*glnA*	75,000	6	ND[e]	ND	ND	*Bacteroides*, *Butyrivibrio*, *Synechocystis*, *Synechococcus*, *Gleocapsa*, *Pseudoanabaena* spp.
GlnT[f]	*glnT*	47,000	8	Heat stable	33	ADP-ribosylation[g]	*Rhizobium*, *Agrobacterium* spp.

[a] K_m for ammonium.
[b] Not reported in cyanobacteria.
[c] In *R. rubrum*, *Streptomyces griseus*, and *Synechocystis* spp.
[d] In *R. leguminosarum* (154).
[e] ND, not done.
[f] Also called GSIII.
[g] In *R. meliloti.*

review, we concentrate on recent research with enterics which has provided considerable insight into the molecular mechanisms that facilitate nitrogen control. We then discuss research with other prokaryotes which is not only demonstrating how widespread the nitrogen regulation (*ntr*) system is but also revealing novel aspects of this fundamental metabolic control system.

NITROGEN ASSIMILATION

In virtually all cells, glutamate and glutamine serve as the key nitrogen donors for biosynthetic reactions and there are two major pathways which facilitate the incorporation of nitrogen into glutamate and glutamine. The most important pathway is the glutamine synthetase/glutamate synthetase (GS/GOGAT) pathway, which is ubiquitous in bacteria. Glutamine synthetase (GS) converts glutamate and ammonia to glutamine, and glutamate synthetase (GOGAT) transfers the amide group from glutamine to 2-ketoglutarate to produce two glutamate molecules. The overall reaction is the production of glutamate from ammonia and 2-ketoglutarate, as shown below.

$$NH_3 + \text{glutamate} + ATP \xrightarrow{GS} \text{glutamine} + ADP + P_i$$

$$\text{Glutamine} + \text{2-ketoglutarate} + NADPH \xrightarrow{GOGAT} 2\ \text{glutamate} + NADP^+$$

Glutamine Synthetase

The common form of GS (GSI) is a dodecameric enzyme with identical subunits of approximately 55 kDa, encoded by *glnA*. The crystal structure of this enzyme revealed that it is composed of 12 identical subunits arranged as two superimposed hexagonal rings that are held together by both hydrophobic interactions and hydrogen bonding between the subunits (271).

GSI enzymes can be subdivided according to whether, and how, they are posttranslationally modified. GSI from the family *Enterobacteriaceae*, *Vibrio alginolyticus* (24), *Thiobacillus ferrooxidans* (14), *Streptomyces cattleya* (233), and *Streptomyces coelicolor* (270) are all subject to posttranslational modification by adenylylation, whereas those from *Bacillus* (51) and *Clostridium* (115, 127) species are not. Adenylylation regulates the catalytic activity of GSI by the covalent addition of an AMP group to a tyrosine residue in each subunit, which progressively inactivates the enzyme. This occurs in response to an increase in the intracellular nitrogen status such that as the cell becomes more nitrogen sufficient, the activity of GSI is progressively reduced. Adenylylation of a subunit of GSI inactivates only that subunit, so that the enzyme can exist in a range of activity states in the cell.

The process of adenylylation is catalyzed by an adenylyltransferase, which is a monomer with a molecular mass of 115 kDa. The adenylyltransferase structural gene *glnE* has so far been cloned and sequenced only from *E. coli* (247), in which it has been located at 67 min on the physical map (182). The physiological role of adenylyltransferase in *Salmonella typhimurium* was studied by using *glnE* mutants (140). These strains show a large growth defect upon shift from nitrogen limitation to medium containing excess ammonium. This is due to the very high catalytic activity of GS after the shift, resulting in a lowering of the intracellular glutamate pool. It was therefore proposed that a major function of adenylylation of GS is to protect the cellular glutamate pool in conditions of ammonia shock and thereby allow rapid growth.

Recent studies have demonstrated a second form of covalent modification of GSI, namely, ADP-ribosylation, which, like adenylylation, occurs in response to ammonia shock. This has so far been found in *Rhodospirillum rubrum*, *Streptomyces griseus*, and *Synechocystis* strain PCC 6803 (197, 227, 264), but the physiological significance of this modification is not known.

glnA genes have been cloned from many different prokaryotes, and the primary amino acid sequence of GSI is highly conserved across a wide range of genera (266). However while GSI is the predominant form of GS in prokaryotes, a number of bacteria are now known to synthesize more than one form of GS (Table 1).

A second form of GS (GSII) was originally identified in *Rhizobium* strains (46) and subsequently in *Agrobacterium* strains (89). GSII is encoded by the *glnII* gene and differs markedly from GSI in that it is an octomer of identical subunits (molecular mass, 36 kDa) and is heat labile. There is some evidence that GSII in *Rhizobium leguminosarum* may be subject to posttranslational modification (162). The occurrence of GSII in plant-symbiotic bacteria and the fact that its amino acid sequence has greater similarity to eukaryotic GS enzymes than to bacterial GSI led to the proposal that it was the result of lateral gene transfer from plants to bacteria (30). However, a detailed phylogenetic analysis of GSI and GSII sequences casts doubt on this hypothesis (224), and GSII has since been found in other genera including *Streptomyces* (17, 139) and *Frankia* (61, 109). The current hypothesis is that the gene originated by gene duplication in preprokaryotes (organisms prior to the eukaryote-prokaryote division) and that GSI was either lost or modified in eukaryote predecessors (138).

GSIII was initially identified in *Bacteroides fragilis* (104), and homologous genes have been identified in *Butyrivibrio fibrisolvens* (95) and *Synechocystis* strain PCC 6803 (209). GSIII is a hexamer with a subunit molecular mass of 75 kDa but is not extensively characterized. The amino acid sequence of GSIII is unlike that of either GSI or GSII, although several conserved regions around the active site have been identified. While GSIII appears to be the only form of GS in the family *Bacteroidaceae*, *Synechocystis* spp. synthesize both GSI and GSIII. Homologs of the *Synechocystis* GSIII gene, *glnN*, were identified by Southern hybridization in *Synechococcus* spp., *Gloecapsa* spp., and *Pseudoanabaena* spp. but not in nitrogen-fixing filamentous species of cyanobacteria (209).

A fourth GS has been identified in *Rhizobium leguminosarum* and *Rhizobium meliloti* and is distinct from the other GS enzymes (38, 49, 223). The nomenclature for this enzyme is confusing, as it is variously called GlnT, in accordance with the structural gene designation (*glnT*), and GSIII, which conflicts with the designation of the *Bacteroides* GS. Likewise, with the proliferation of GS enzymes now recognized in bacteria, the current genetic nomenclature for GS structural genes is becoming somewhat confusing. It would undoubtedly benefit from agreement on a uniform system in which the genetic designation reflected the type of GS that the gene encodes, i.e., *glnA* should always encode a GSI enzyme, etc.

The GlnT enzyme is an octomer, like GSII, with a subunit molecular mass of 47 kDa, but unlike GSII, it is stable at elevated temperatures. The enzyme has a much higher substrate K_m for glutamate and ammonia than does either GSI or GSII, and consequently it has a much lower biosynthetic activity (67), which led Shatters et al. (223) to question whether the primary function of this protein was the synthesis of glutamine. However, there is no evidence of any other enzymatic function associated with the protein, and its primary sequence is closer to that of GS enzymes than to other glutamine-binding enzymes such as glutaminases or amidotransferases. A homolog of *glnT* has also been found in *Agrobacterium tumefaciens* (215). In *R. meliloti*, GlnT can be posttranslationally modified by ADP-ribosylation (150). The modification takes place on an arginine residue and inhibits GlnT activity, but its physiological role is unknown.

Glutamate Synthase

Glutamate synthase (previously known as glutamine amide-2-oxoglutarate aminotransferase [GOGAT]) has two nonidentical subunits which in *E. coli* have molecular masses of 53 and 135 kDa and are encoded by the cotranscribed *gltBD* genes (190). In *E. coli*, the *gltBD* genes are members of the Lrp (leucine-responsive regulatory protein) regulon, and Lrp plays a role in repression of GOGAT synthesis in rich medium in which nitrogen is present in the form of amino acids and nucleic acid bases as well as ammonia (65). The *glt* locus has also been characterized in *Azorhizobium sesbaniae* (56, 103), *R. meliloti* (144) and *Azospirillum brasilense* (163). In *Bacillus subtilis*, the GOGAT subunits are encoded by *gltA* and *gltB*, which are genetically linked but noncontiguous (25).

Glutamate Dehydrogenase

In many organisms, the GS/GOGAT pathway, which allows the assimilation of ammonia present in the medium at concentrations lower than 0.1 mM, is the only pathway for utilization of ammonia. However, in other bacteria, including the enterics, an alternative route of assimilation is present, namely, by means of glutamate dehydrogenase (GDH), which is encoded by the *gdhA* gene and in *E. coli* is a hexamer of identical subunits each with a molecular mass of 50 kDa. GDH catalyzes the reductive amination of 2-ketoglutarate by ammonia to give glutamate in an NADPH-dependent reaction. However, owing to the relatively high K_m of the enzyme (around 1 mM), the enzyme is rather ineffective in nitrogen assimilation for cells growing under ammonia limitation.

$$NH_3 + \text{2-ketoglutarate} + NADPH \xrightarrow{GDH} \text{glutamate} + NADP^+$$

Ammonium Transport

There is considerable evidence for the rapid diffusion of ammonia across cytoplasmic membranes, and hence until recently the concept of active transport of ammonium ions has received relatively little attention. However, the large ammonia gradients which can occur across bacterial membranes argue strongly for an active ammonium transport (Amt) system, and there is some evidence for such systems in about 50 prokaryotic species (for reviews, see references 13, 128, and 129). In most cases, Amt activity is repressed in the presence of high extracellular ammonium concentrations, and studies with the ammonium analog [^{14}C]methylammonium in *E. coli* indicated that Amt expression is controlled by the Ntr system (118, 222).

Potential Amt$^-$ mutants have been found in *E. coli* (69, 116, 117) and in *K. pneumoniae* (34). In *E. coli*, the *amtA* gene has been mapped to 95.8 min and has been cloned and sequenced. The AmtA protein is proposed to be a 27-kDa peripheral membrane protein, but its role in ammonium transport is still unknown (13). Recently, however, genes required for high-affinity ammonium transport have been cloned from *Saccharomyces cerevisiae* (165) and from *Arabidopsis thaliana* (187). These genes encode highly homologous membrane proteins of around 54 kDa, which were found to have significant sequence similarity to several bacterial and animal proteins of previously unknown function. Included in this family are the nitrogen-regulated *nrgA* gene of *Bacillus subtilis* (269) and an open reading frame (ORF) from *Rhodobacter capsulatus* (120), and it will be of interest to see whether ammonium transport in bacteria is facilitated by the products of such genes.

NITROGEN REGULATION (*ntr*) SYSTEM IN THE FAMILY *ENTEROBACTERIACEAE*

Ammonia is almost invariably the preferred source of nitrogen for bacterial growth in that it supports a higher growth rate than any other nitrogen source. However, bacteria frequently have to utilize a wide range of alternative nitrogen sources, and to accomplish this they are capable of synthesizing numerous proteins for the uptake and subsequent metabolism of nitrogenous compounds. The synthesis, and in some cases the activity, of these proteins is tightly regulated in concert with the availability of their substrates, and this regulation, which was first recognized in the pioneering work of Magasanik and coworkers on histidase synthesis in *Klebsiella aerogenes* (199, 200), is now termed nitrogen control. The biochemical and genetic mechanisms that facilitate nitrogen control are undoubtedly most clearly understood in the enteric bacteria, although elements of the system are now recognized in many other bacterial groups.

The global nitrogen regulatory (*ntr*) system is composed of four enzymes: a uridylyltransferase/uridylyl-removing enzyme (UTase/UR), encoded by the *glnD* gene, a small trimeric protein, P_{II}, encoded by *glnB*, and a two-component regulatory system composed of the histidine protein kinase NtrB and the response regulator NtrC (Fig. 1). The recognition of the roles of these four proteins derived primarily from two convergent

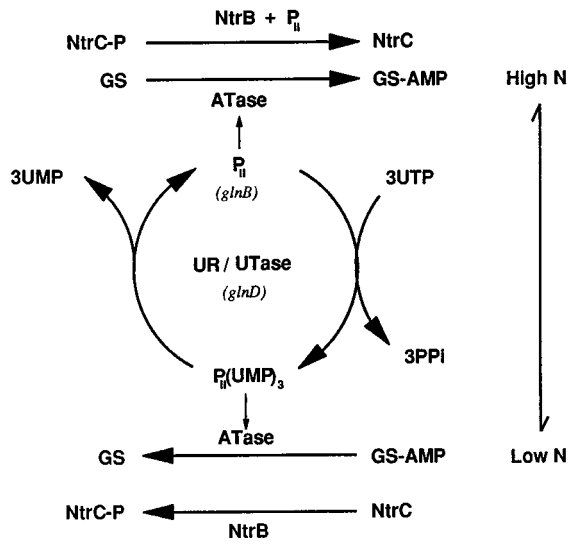

FIG. 1. Schematic model for the regulation of the activities of GS and NtrC protein in response to nitrogen status. UTase (*glnD* product) catalyzes the uridylylation and deuridylylation of P_{II} (*glnB* product). Adenylyltransferase catalyzes the adenylylation and deadenylylation of GS. NtrB protein catalyzes the phosphorylation and dephosphorylation of NtrC protein.

fields of study: the genetic analysis of the transcriptional regulation of the synthesis of GSI, histidase, and nitrogenase (for reviews, see references 169 and 208) and the elegant studies of Stadtman et al. on regulation of GSI activity by adenylylation (229). Studies on GS adenylylation (26) led to the recognition that adenylyltransferase (ATase) activity is regulated by UTase/UR and P_{II} (1, 164) such that when cells are nitrogen limited, UTase covalently modifies P_{II} by addition of a UMP group at a specific tyrosine residue on each subunit of the protein and the resultant uridylylated form of P_{II} promotes deadenylylation of GS by ATase. Conversely, under nitrogen-sufficient conditions, the uridylyl-removing activity of GlnD predominates and the deuridylylated P_{II} promotes adenylylation of GS by ATase. These studies lead to the conclusion that UTase/UR and P_{II}, acting together, can provide a mechanism for sensing the intracellular nitrogen status.

Much of our initial understanding of transcriptional control by the enteric *ntr* system came from studies of the regulation of the GSI structural gene, *glnA*. In enterics, *glnA* is part of a complex operon, *glnAntrBC* (4, 133, 176, 191), in which *ntrB* and *ntrC* encode one of the paradigms of bacterial two-component regulatory systems. Such systems, of which dozens have been described in prokaryotes and which are now also being recognized in eukaryotes (237), typically comprise a sensory histidine protein kinase (e.g., NtrB) and a response regulator (e.g., NtrC), which is often a DNA-binding protein (232).

The *glnA* gene is expressed from two tandem promoters *glnA*p1 and *glnA*p2, whereas the downstream *ntrBC* genes are expressed either by readthrough from the *glnA* promoters or from a separate promoter, p*ntrBC*, situated between *glnA* and *ntrBC* (55, 157, 207, 245). Under nitrogen-sufficient conditions, *glnA* is expressed from *glnA*p1, which is transcribed by the major vegetative form of RNA polymerase, $E\sigma^{70}$. Most of these transcripts terminate at a Rho-independent terminator just downstream of *glnA*, and expression of *ntrBC* occurs primarily from p*ntrBC*. Under nitrogen-limiting conditions, the binding of NtrC to sites that overlap *glnA*p1 serves to repress expression from *glnA*p1 while activating transcription from *glnA*p2 by RNA polymerase containing the sigma factor σ^N. This transcription is at a considerably elevated level compared with that from *glnA*p1, and a proportion of these transcripts read through into *ntrBC*. The consequence of this organization is to provide a low level of *glnA ntrBC* transcription under conditions of nitrogen sufficiency, resulting in an estimated intracellular concentration of NtrC of 5 dimer molecules per cell (206) and a marked elevation in transcription, approximately 14-fold, when fixed nitrogen is limiting. The mode of action of each component of the enteric *ntr* system has now been analyzed in some considerable detail by both genetic and biochemical approaches, which are summarized below.

Uridylyltransferase (GlnD)

The enzyme activities responsible for removal of UMP from P_{II} (UR) and attachment of UMP to P_{II} (UTase) were purified from *E. coli* and shown to copurify as a protein of around 100 kDa through a variety of procedures (1, 82, 91). The availability of fixed nitrogen to the cell is reflected in the intracellular ratio of glutamine to 2-ketoglutarate such that ammonia sufficiency is reflected in a high glutamine/2-ketoglutarate ratio and ammonia deficiency lowers that ratio. Studies on the effects of a number of metabolites on the activities of both UTase and UR indicated that many metabolites affect one or other activity but that most notably UTase activity was stimulated by 2-ketoglutarate and ATP and inhibited by glutamine. Consequently, these observations led to a model in which the ratio of glutamine to 2-ketoglutarate determines the uridylylation state of P_{II} (64).

The UTase/UR structural gene, *glnD*, has been cloned and sequenced from *E. coli* and *K. pneumoniae*, and the two genes encode very similar proteins (84% identical) with molecular masses of 102 kDa. In each case, *glnD* is located immediately downstream of the *map* gene, encoding the N-terminal methionine aminopeptidase, and immediately upstream of *dapD*, an enzyme involved in the biosynthesis of lysine (62, 246, 247). Partial DNA sequence indicates that the same situation is found in *Salmonella typhimurium* (18, 174, 212), in which *map* is designated *pepM*. RNase protection and primer extension mapping demonstrated that in *E. coli* these three genes are separately transcribed. There is some disagreement in the literature on the transcriptional control of *glnD*, with two reports suggesting that the gene is nitrogen regulated under certain conditions (122, 211) and another finding no evidence for such regulation (247). Likewise, independent studies have reported two different transcript starts (122, 247). Analysis of the *glnD* upstream region by Kamberov et al. (122) identified two potential binding sites for the transcriptional regulatory protein Nac and a possible σ^N-dependent promoter, but this shows a very poor match to the consensus σ^N promoter sequence.

P_{II} (GlnB)

The P_{II} structural gene, *glnB*, has been cloned and sequenced from *E. coli* (228, 250) and *K. pneumoniae* (107) and is predicted to encode a polypeptide of 12.4 kDa. The site of uridylylation is identified as Tyr-51 (210, 228). Two classes of *glnB* mutation have been described. The first class causes glutamine auxotrophy and has been attributed to the production of a form of P_{II} which cannot be uridylylated. This promotes adenylylation of GS under all conditions as well as preventing phosphorylation of NtrC and consequent activation of the *glnA*p2 promoter. One such *glnB* allele has been sequenced and shown to alter the residue immediately adjacent to Tyr-51, namely, Glu-50, to Lys-50 (107). The second class of *glnB* mutation encodes null mutants which produce no P_{II} or inac-

tive P_{II}. Such mutations arise as suppressors of the first class of *glnB* alleles or via suppression of mutations in *glnD* or *ntrB* (27, 160).

Characterization of P_{II} through the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and sedimentation centrifugation originally suggested that it was a tetramer of four identical subunits (1). However, recent success in obtaining crystals of P_{II} indicates that it is in fact a trimer, and this has been confirmed independently by analytical equilibrium centrifugation (36, 50, 249). Most of the residues in the trimer pack into a squat barrel approximately 50 Å (5 nm) in diameter and 30 Å (3 nm) high. The most distinctive features of the trimer are the three loops which protrude from one of the flat surfaces of the trimer. Each of these loops is an eight-residue stretch with Tyr-51 at its apex. The hydroxyl group of Tyr-51 is some 13 Å (1.3 nm) above the surface of the molecule, making it readily accessible for uridylylation. In principle, other residues within this protruding loop could also offer sites of covalent modification, and in other members of the P_{II} protein family this apparently occurs (see below). A putative ATP-binding site has also been identified at the C terminus of P_{II} on the opposite face of the molecule to the exposed tyrosine loop (50).

Recent studies have shown that while UTase is capable of binding glutamine, P_{II} can bind ATP, 2-ketoglutarate, and glutamate (50, 123, 149). The binding of P_{II} to NtrB to stimulate the NtrB phosphatase activity is activated when P_{II} binds ATP and either 2-ketoglutarate or glutamate (123). Consequently, under conditions of nitrogen limitation, 2-ketoglutarate seemingly induces a conformational change in P_{II} to put it in an optimal conformation for uridylylation, and under conditions of nitrogen sufficiency, UTase binds glutamine, which inhibits the uridylylation reaction and switches the protein to a conformation which preferentially deuridylylates P_{II} (50, 123). The uridylylation of the trimeric P_{II} by UTase prevents the binding of P_{II} to NtrB (123). In vitro, uridylylation is a noncooperative reaction in which partially modified P_{II} species accumulate and these trimers are in turn partially active in stimulating dephosphorylation of NtrC (9).

Upstream of the *E. coli glnB* gene, and cotranscribed with it, are two ORFs, ORF1 and ORF2. ORF1 is not homologous to any other known proteins, but ORF2 shows strong homology to the σ^N-dependent activator protein, NtrC, both in its amino-terminal domain with conservation of the normally phosphorylated aspartate residue and in the central domain containing the ATP-binding site (148, 247). Despite this intriguing homology, no phenotype was detected in a strain deleted for ORF2 and no physiological role has been assigned to either protein (148).

The major *glnB* transcripts are initiated at two sites 34 and 101 bp upstream from the translation start site, and a binding site for PurR, which is responsible for repression of enzymes required for purine nucleotide biosynthesis, is located immediately downstream of the second of these sites (101, 148, 247). PurR exerts about a twofold repression of *glnB* transcription, but the functional significance of this regulation is not clear (101). A minor *glnB* transcript is initiated upstream of ORF1 and ORF2. None of the transcripts is regulated in response to nitrogen status (148, 247).

Studies by Bueno et al. (27) demonstrated that in *E. coli* the phenotype of a *glnD*::Tn*10* Δ*glnB* strain was different from that of a Δ*glnB* strain, suggesting that UTase might have another target in addition to P_{II}. Recent studies with *E. coli* have now identified a second copy of *glnB* in this organism (248). The gene encodes a protein with 67% identity to P_{II}, but unlike *glnB*, the gene encoding the P_{II} homolog is regulated in response to N status such that its expression is elevated under nitrogen-limiting conditions. The role of this P_{II} homolog in enteric nitrogen control has yet to be determined.

NtrB

Histidine protein kinases typically comprise two domains, a sensor domain, which is normally N terminal and in the case of membrane-bound sensors has one or more membrane-spanning helices, and a C-terminal kinase domain. The kinase domain includes three highly conserved motifs, an amino-terminal motif including a histidine residue that is an autophosphorylation site, a motif with a conserved asparagine residue of unknown function, and a carboxy-terminal motif containing a potential nucleoside triphosphate-binding site (232). NtrB is a 36-kDa dimeric protein that conforms precisely to this model, although since it is a cytoplasmic protein, it has no membrane anchor (157, 213). The cytoplasmic signal which modulates the activity of NtrB is P_{II}, which in the form of P_{II}-UMP signals nitrogen limitation and when unmodified signals nitrogen excess.

Under nitrogen-limiting conditions, NtrB catalyzes the phosphorylation and consequent activation of its partner response regulator, NtrC (124, 184, 261). NtrB is initially autophosphorylated on residue His-139 in an ATP-dependent reaction (183). This reaction occurs within the dimer by a *trans*, intersubunit mechanism in which one subunit binds ATP and phosphorylates the other subunit (186). Alteration of the phosphorylation site, e.g., His-139 to Asn-139, or deletion of the C-terminal 59 amino acids prevents autophosphorylation, and the C-terminal deletion impairs ATP binding, although the precise site of ATP binding on NtrB is not yet known (186).

Under nitrogen-sufficient conditions, P_{II} in its nonuridylylated form interacts with NtrB such that NtrB now catalyzes dephosphorylation and consequent inactivation of NtrC. The positive and negative regulatory functions of NtrB can be separated genetically so that alteration of many of the highly conserved residues such as His-139 impair the kinase but not the phosphatase function of NtrB (7). Whereas the phosphatase activity of wild-type NtrB is observed only in the presence of P_{II}, certain mutant forms of NtrB, e.g., His-139-to-Asn, show phosphatase activity in the absence of P_{II} (121). The phosphatase activity of such a mutant is stimulated by P_{II} and by ATP, suggesting that P_{II} is not directly involved in catalysis of the phosphatase activity but rather in its regulation. A truncation eliminating the N-terminal 110 amino acids of NtrB has kinase activity but lacks phosphatase activity, an observation consistent with the domain structure of NtrB, which suggests that the N-terminal domain regulates the kinase activity of the C-terminal domain. Hence, the current working hypothesis is that the N-terminal domain of NtrB acts as the receptor for P_{II} (121). This has not yet been formally demonstrated, but the occurrence of many signal transduction mutations within the N-terminal domain supports this hypothesis (6).

The signal transduction system from UTase through to the dephosphorylation of phosphorylated NtrC (NtrC-P) has now been reconstituted in vitro such that in the presence of glutamine, UTase/UR catalyzes the removal of uridylyl groups from P_{II}, which in turn stimulates dephosphorylation of NtrC by NtrB (9).

NtrC

The response regulator NtrC is a dimeric protein with a subunit molecular mass of 55 kDa. It is characteristic of the σ^N (σ^{54})-dependent activator proteins (141, 181) and has three distinct domains (57, 206). The N-terminal domain of some 12

kDa is characteristic of response regulators and constitutes the receiver domain with which the sensor protein interacts to phosphorylate a conserved aspartate residue (Asp-54) (124, 131, 184, 217, 261). Only the phosphorylated form of the protein is competent to activate transcription (185), and substitutions of other residues for Asp-54 significantly impair the response to nitrogen deprivation (178). Phosphorylation of NtrC stimulates DNA binding (260) but is not required for DNA binding per se; rather, it is required to induce oligomerization of NtrC dimers at upstream activator sequences (173a), which are composed of at least two NtrC-binding sites.

The three-dimensional solution structure of the NtrC N-terminal domain has been solved by nuclear magnetic resonance spectroscopy and compared with the previously determined structure of the response regulator CheY (253). CheY is a single-domain protein that interacts with targets in the flagellar motor complex to control the direction of flagellar rotation. By contrast, the downstream target of the NtrC receiver domain is within the same protein, and hence differences in the structures of the CheY and NtrC domains may indicate regions that are important for interaction with their respective targets. The NtrC and CheY domains have very similar folds, with the only significant difference being the relative position of one α-helix (helix 4). Evidence for a functionally important role for helix 4 comes from identification of "constitutive" NtrC mutants, i.e., proteins that have some ability to activate transcription without being phosphorylated. Two of the three "constitutive" amino acid substitutions so far identified in the receiver domain of NtrC (Asp-86-to-Asn and Ala-89-to-Thr) are in helix 4 (253), the third (Asp-54-to-Glu) being at the phosphorylation site (131).

The central domain of approximately 240 amino acid residues is characteristic of the family of σ^N-dependent activator proteins. It contains a conserved nucleoside-binding site and is considered to be the domain which interacts with σ^N-containing RNA polymerase ($E\sigma^N$) to activate transcription by that enzyme. NtrC has an ATPase activity that is essential for open-complex formation by $E\sigma^N$ (11, 256a, 259). This ATPase activity is stimulated by DNA binding and by phosphorylation at Asp-54.

DNA binding of the dimer is mediated by the C-terminal domain, which contains a typical helix-turn-helix motif (43). This motif allows recognition by NtrC of the upstream activator or enhancer sequences, which are typically located some 100 bp upstream of the promoter sequence at which $E\sigma^N$ binds. The function of this enhancer is to facilitate oligomerization of the protein to form a complex containing at least two dimers which is required for transcriptional activation (130, 198) and to tether NtrC at high local concentration near the promoter and thereby increase its frequency of contact with $E\sigma^N$ (234, 256). A model of the structural components of the C-terminal domain, based on observed homology to the homodimeric Fis protein, has been proposed (130). This model proposes that the C-terminal domain also contains the major dimerization determinants within the protein, but conflicting data (70a) suggest that the N-terminal "receiver" domain of NtrC is responsible for dimerization. In the latter model, it is proposed that in unphosphorylated NtrC the ability of the N-terminal domain to dimerize is masked by interaction with the central domain and that phosphorylation relieves this inhibition, thereby inducing activation by dimerization.

By virtue of its DNA-binding properties, NtrC can also act as a transcriptional repressor, and in enteric bacteria it functions in this mode at both *glnA*p1 and p*ntrBC* when cells are subject to nitrogen limitation and *glnA ntrBC* transcription is primarily from *glnA*p2 (207, 245, 257).

NtrC phosphorylation by acetyl phosphate. In vitro studies with the purified chemotaxis response regulator CheY showed that small molecules containing high-energy phosphoryl groups including acetyl phosphate, carbamyl phosphate, and phosphoramidate can donate phosphoryl groups to CheY (153). Subsequent studies demonstrated that NtrC could be phosphorylated by acetyl phosphate and that the NtrC-P so formed had all the properties of NtrC-P produced by the action of NtrB (70). During growth on glucose-glutamine medium, synthesis of GS is normal in phosphotransacetylase (*pta*) mutant strains unable to synthesize acetyl phosphate but is eliminated in strains also lacking NtrB. However when *pta ntrB* strains are grown on acetate (conditions which should lead to accumulation of acetyl phosphate), GS synthesis is equivalent to that in the wild type grown on glucose-glutamine. Hence, acetyl phosphate could act as a positive regulatory signal for activation of NtrC, and similar results have been reported for activation of the phosphate regulon activator PhoB by acetyl phosphate (254).

A possible physiological role for activation of NtrC by acetyl phosphate may be to facilitate the shift down from nitrogen-rich to nitrogen-poor conditions, under which the cells initially have very few molecules of NtrB. If acetyl phosphate levels are elevated under nitrogen-limiting conditions, this would provide an NtrB-independent route to stimulate transcription from *glnA*p2 and hence to raise levels of both NtrB and NtrC, thereby priming the system under conditions where the regulators are otherwise present in very small amounts (70). However, measurements of acetyl phosphate concentrations in wild-type and *ntrB* mutant strains of *E. coli* (201) suggest that all the data of Feng et al. (70) cannot be accounted for by this model and that other acetate derivatives might be involved.

Genes Regulated by the *ntr* System

In enteric bacteria, the genes currently recognized as transcriptionally regulated by NtrBC include *glnA ntrBC*; genes encoding transport systems for glutamine (*glnHPQ*) (39, 189), arginine (*argT*) (218), and histidine (*hisJQMP*) (218); the genes required for nitrate and nitrite assimilation (*nasFEDCBA*) (94); the nitrogen fixation regulatory genes *nifLA* of *K. pneumoniae* (66, 175); and the nitrogen regulation gene (*nac*) of *K. aerogenes* (156).

OTHER NITROGEN CONTROL GENES IN ENTERIC BACTERIA

nac

In *K. aerogenes*, a subset of nitrogen-regulated enzymes including histidase (*hut*), proline oxidase (*put*), urease (*ure*), GDH (*gdh*), and GOGAT (*gltBD*) are under control of the *nac* gene product (156, 220). Nac is a dimer with a subunit molecular mass of 32 kDa that belongs to the LysR family of regulatory proteins (221). However, Nac appears to require no coeffector and does not respond to N status itself; instead, *nac* expression is σ^N dependent and is nitrogen regulated by NtrBC (156). Consequently, Nac is only synthesized under nitrogen-limiting conditions when it can act either to activate genes such as *hut*, *put*, and *ure* or to repress expression of *gdh* and *gltBD*. The purification of Nac and studies of its interactions with the *hutU*p, *ureD*p, and *putP*p1 promoter regions have allowed identification of the Nac-binding consensus sequence, and subsequent database searches have suggested that Nac may also be involved in regulation of *E. coli codBA* (encoding cytosine utilization) and *E. coli asnAC* (encoding asparagine synthesis) (96).

The *nac* gene was first described in *K. aerogenes* (20) and is apparently present in *E. coli* (19) and *K. pneumoniae* (171) but is absent from *Salmonella typhimurium*. The *hut* genes of *S. typhimurium* are not activated in response to nitrogen limitation unless *nac* is introduced into the organism (22). Nac regulation appears to be relatively insignificant in *E. coli*, in which it does not affect *gdh* at all and only affects *put* slightly. The precise need for Nac in a regulatory sense is not at all clear, but it appears to provide a means of coupling the expression of a number of genes that are σ^{70} dependent to the otherwise σ^{N}-dependent *ntr* system (19). To date, Nac has not been identified in any nonenteric organism.

gltF

Another gene that has been implicated in nitrogen regulation is *gltF*, which lies downstream of *gltBD* and is apparently part of the same operon. *gltF* has so far been found only in *E. coli* and *K. pneumoniae* (32, 33, 137). Mutations in *gltF* prevent normal induction of histidase and do not show the glutamate-dependent repression of the *glt* operon seen in wild-type cells. GltF encodes a 26-kDa polypeptide with a predicted signal sequence, suggesting that it may be a periplasmic protein. A proposed homology of GltF to histidine protein kinases is very weak, and the function of the protein is presently unknown (33).

rpoN Is Not a Nitrogen Regulation Gene

As discussed above, NtrC activates transcription by the σ^{N}-containing form of RNA polymerase. Historically, σ^{N} has been associated with nitrogen regulation, because its structural gene (then designated *glnF*) was originally identified by isolation of glutamine auxotrophs in *S. typhimurium* (90). Later, the gene was renamed *ntrA* when it was recognized as playing a role in the expression of other nitrogen-regulated genes (168), and it was finally designated *rpoN* when it was shown to encode a sigma factor (111). However, it should be understood that *rpoN* is not per se a nitrogen regulation gene. Neither the expression nor the activity of σ^{N} is normally subject to nitrogen control, an exception being *Rhodobacter capsulatus nifR4*, in which expression is nitrogen regulated (45). Furthermore, in a number of cases, σ^{N} is required for expression of genes that are not subject to nitrogen control (170), e.g., hydrogenase genes in *E. coli* (155) and xylene degradation genes in *Pseudomonas putida* (132).

OCCURRENCE OF *ntr* GENES IN OTHER BACTERIAL GENERA

Studies of nitrogen control have been extended to many bacterial genera, and there is now evidence for the presence of an analagous Ntr system, or at least elements of one, in many different organisms (Table 2). In some cases, the evidence is based solely on sequences of genes encoding Ntr proteins, but in others, it extends to mutational analysis.

glnD

There are only two reports of UTases outside the enterics, one being encoded by the *Azotobacter vinelandii nfrX* gene (44) and the other being encoded by a gene in the recently published sequence of the complete genome of *Haemophilus influenzae* (76).

The *nfrX* gene can complement mutations in *glnD* in *E. coli*, *K. aerogenes*, and *K. pneumoniae*, and likewise, enteric *glnD* genes complement *A. vinelandii nfrX* mutations. Furthermore, the predicted NfrX amino acid sequence shows 41% sequence identity to that of GlnD from *E. coli* or *K. pneumoniae*. Mutations in *nfrX* prevent expression of the nitrogen fixation (*nif*) genes in *A. vinelandii* by an unknown mechanism. The Nif^{-} phenotype of *nfrX* mutants can be suppressed by mutations in the *nif* regulatory gene *nifL* (44), suggesting that NifL is a direct or indirect target for NfrX, but the relationship between these proteins and the mechanism of nitrogen sensing by NifL remains unclear (see below). Unlike *glnD* mutants in enterics, *nfrX* mutants are not impaired in nitrate assimilation or GS adenylylation (44). Nevertheless, homologs of *ntrBC* are found in *A. vinelandii*, and growth on certain nitrogen sources such as nitrate is *ntrBC* dependent (240). Hence, no link has yet been established between NfrX and NtrBC in *A. vinelandii*, and there remains the possibility that this organism has a second *glnD*-like gene or senses nitrogen by a different system.

The *H. influenzae* genome sequence includes homologs of both *glnD* and *glnB* (see Fig. 2), but homologs of *ntrB* and *ntrC* have not been identified. *H. influenzae glnD* encodes a protein with 40% sequence identity to the enteric GlnD, and homology is distributed throughout the length of the protein.

Although no UTase proteins or their cognate genes have yet been characterized in the rhizobia, extracts of *Rhizobium leguminosarum* have been shown to modify P_{II} by uridylylation (41) and a cosmid from *R. leguminosarum* has been shown to complement *A. vinelandii nfrX* mutants (100); therefore a UTase can be inferred to be present in this organism.

TABLE 2. Nitrogen regulation genes/proteins in different bacterial genera

Species	Gene and protein status[a]:					
	Utase/UR	P_{II}	P_{II}*[b]	NtrB	NtrC	Other
Escherichia coli	p	p	s	p	p	*nac*
Salmonella typhimurium	s	g		p	p	
Klebsiella pneumoniae	s	s	g	p	p	
Klebsiella aerogenes	g	g		g	g	*nac*
Azotobacter vinelandii	s	s		s	s	
Pseudomonas putida		p				
Proteus vulgaris				s	s	
Vibrio alginolyticus				s	s	
Thiobacillus ferrooxidans				s	s	
Rhizobium meliloti					s	
Rhizobium leguminosarum		s		s	s	*nifR3*
Bradyrhizobium japonicum		s		s	s	
Azorhizobium caulinodans		s		s	s	*ntrXY*
Agrobacterium tumefaciens					s	
Azospirillum brasilense		s	g	s	s	*nifR3*
Rhodobacter capsulatus		s		s	s	*nifR3*, *nadE*
Haemophilus influenzae	s	s				
Streptomyces coelicolor						*glnR*
Bacillus subtilis		s				*glnR*[c], *outB*[d]
Cyanobacteria		s				*nctA*
Archaeobacteria		s				

[a] g, gene identified; s, gene sequenced; p, protein purified.
[b] P_{II}* denotes the protein encoded by a second copy of *glnB*; the distinction between P_{II} and P_{II}* is presently arbitrary.
[c] The *glnR* gene of *B. subtilis* is not equivalent to *S. coelicolor glnR*.
[d] *B. subtilis outB* encodes a protein with marked homology to the *nadE* product.

glnB

Homologs of P_{II}. (i) Transcriptional regulation. A total of 14 genes encoding potential members of the GlnB family have been sequenced, and 9 of these encode polypeptides which have a conserved tyrosine residue at the uridylylation site and

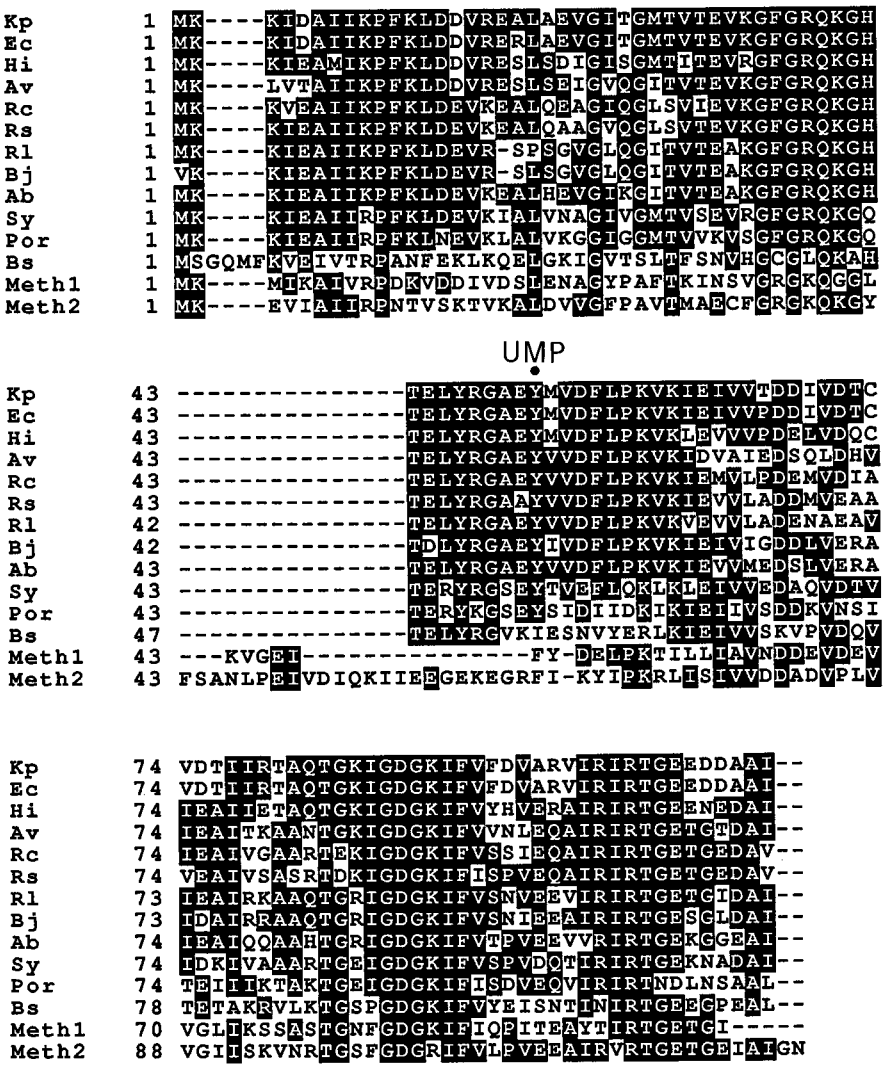

FIG. 2. Alignment of the protein sequences of the P_{II} and P_{II}-like proteins identified to date. The tyrosine which is the site of uridylylation is marked by UMP. Kp, *Klebsiella pneumoniae*; Ec, *Escherichia coli*; Hi, *Haemophilus influenzae*; Av, *Azotobacter vinelandii*; Rl, *Rhizobium leguminosarum*; Bj, *Bradyrhizobium japonicum*; Ab, *Azospirillum brasilense*; Rc, *Rhodobacter capsulatus*; Rs, *Rhodobacter sphaeroides*; Bs, *Bacillus subtilis*; Sy, *Synechococcus* strain PCC 7942; Por, *Porphyra purpurea*; Meth1 and Meth2, *Methanococcus thermolithotrophicus* (products of the adjacent ORF105 and ORF128). Conserved residues are shown as white letters on a black background.

appear to be functional homologs of the enteric P_{II} proteins (Fig. 2). The initial characterization of P_{II} was with proteins purified from *E. coli* and *Pseudomonas putida* (1), but the *P. putida glnB* gene has yet to be characterized. Unlike the enteric *glnB* genes, *A. vinelandii glnB* is the first gene of an operon (*glnB nrgA*) in which *nrgA* encodes a protein very similar to that encoded by *B. subtilis nrgA* (125). However, in *B. subtilis*, the gene arrangement is *nrgAB*, with *nrgB* encoding a P_{II} homolog (see below). In *R. leguminosarum*, *Bradyrhizobium japonicum*, *Azospirillum brasilense*, *Rhodobacter capsulatus*, and *Rhodobacter sphaeroides*, the transcriptional organization is different, with *glnB* adjacent to *glnA* and at least partly cotranscribed in a *glnBA* operon (42, 52, 81, 167, 275). The reason for this linkage is not altogether clear, and the regulation of the *glnBA* operon varies in different organisms.

In *B. japonicum*, although *glnB* and *glnA* are adjacent, little cotranscription is detected. The *glnB* gene is transcribed from two promoters: a σ^{70}-like promoter (*glnB*p1) that is expressed under conditions of nitrogen sufficiency and is repressed by NtrC, and a σ^{N}-dependent promoter (*glnB*p2) that is activated by NtrC under conditions of nitrogen limitation. The coordinated effects of these promoters result in a fairly constant level of *glnB* transcription regardless of the nitrogen status (167). The *glnA* gene is expressed from a σ^{70}-like promoter at an essentially constitutive level.

The *glnBA* promoter sequences of *R. capsulatus* and *R. sphaeroides* are quite similar, suggesting similar regulation in each organism. As in *B. japonicum*, the *R. capsulatus glnB* gene is transcribed from tandem promoters, one repressed (*glnB*p1) and one activated (*glnB*p2) by NtrC. However despite being activated by NtrC, neither *R. capsulatus glnB*p2 or the equivalent promoter in *R. sphaeroides* contains a $-24,-12$ sequence characteristic of σ^{N}-dependent promoters (81, 275).

In *R. leguminosarum*, promoters are located upstream of both *glnB* and *glnA*. The *glnB* promoter sequence has a high level of homology to other σ^{N}-dependent promoters, but no clear NtrC-binding sites are present upstream and the activator of this promoter is not known. Furthermore, the 1.8-kb *glnBA* transcript initiated at this promoter is not markedly regulated in response to changing nitrogen source (37). The *glnA* pro-

moter does not contain a consensus sequence characteristic of other previously described promoters, although it shares some homology with the *B. japonicum glnA* promoter sequence (31). The 1.5-kb *glnA* transcript is also not regulated in response to the nitrogen source (37, 216), and it would appear that in both *R. leguminosarum* and *B. japonicum*, GSI is regulated primarily at the level of enzymatic activity, i.e., by adenylylation.

The *A. brasilense glnBA* operon is similar to that in *R. leguminosarum* in that two transcripts, one for *glnBA* and one for *glnA* alone, are detected. As in *B. japonicum*, there are two *glnB* promoters, a σ^{70}-like promoter (*glnB*p1) that is expressed under conditions of nitrogen sufficiency and a σ^{N}-dependent promoter (*glnB*p2) that is activated under conditions of nitrogen limitation. In this case, *glnB* transcription is elevated about fivefold under conditions of nitrogen limitation. However, expression of *glnB* is not NtrC dependent (52, 54), and it is proposed that activation is by an NtrC homolog. Surprisingly, *glnA* expression is lower under conditions of nitrogen limitation than of nitrogen sufficiency.

(ii) Phenotypes of *glnB* mutants. A variety of phenotypes are observed for *glnB* mutants in different organisms, reflecting both the complex functions of P_{II} and the transcriptional organization of the gene in some cases. Knockout mutations in *R. leguminosarum glnB* result in constitutive expression of NtrC-dependent promoters, e.g., *glnII*, as would be expected in a model in which P_{II} can no longer interact with NtrB to promote the dephosphorylation of NtrC and hence NtrC is always phosphorylated. However, these mutations also result in the inability to utilize nitrate as the sole nitrogen source, which conflicts with a model based on the enteric *ntr* system, as expression of nitrate reductase would then be expected to be constitutive (5).

A *glnB*::*kan* mutation in *R. capsulatus* encodes a glutamine auxotroph as a consequence of transcriptional polarity on *glnA* (136), while point mutations in *glnB* were isolated as allowing *nif* gene expression in the presence of fixed nitrogen (134, 135). These mutations presumably inactivate P_{II}, thus allowing constitutive NtrC-dependent expression of *nifA*, the *nif*-specific activator gene. Inactivation of *glnB* in *A. brasilense* also causes constitutive expression, in this case of *glnBA*, indicating that P_{II} autoregulates its own expression, presumably by regulating the activity of the unidentified NtrC homolog responsible for activation of *glnBA* transcription (54).

Other members of the GlnB family. The remaining members of the GlnB family include those P_{II}-like proteins that do not contain a homolog of Tyr-51 or are known to be modified by forms of covalent modification other than uridylylation. They include the product of the *Synechococcus* strain PCC 7492 *glnB* gene, which is subject to covalent modification by serine phosphorylation (77), probably, it has been suggested, at Ser-49 (36). The *Synechococcus* P_{II}-modifying system responds to the activity of ammonium assimilation via the GS/GOGAT pathway and also to the state of CO_2 fixation (78). A P_{II}-deficient mutant of *Synechococcus* strain PCC 7492 is highly pleiotropic, affecting GS levels, uncoupling photosynthetic nitrate reduction from CO_2 fixation, and impairing regulation of methylammonium uptake. Hence, in *Synechococcus* strains, P_{II} seems to be involved in mediating the coordination of nitrogen and carbon assimilation (78).

A similar protein in *Synechococcus* strain PCC 6301 was originally characterized as a phosphorylated 13-kDa protein, the phosphorylation of which was increased in a light regimen that favors photosystem II and decreased in a light regimen that favors photosystem I (99). This led to a model in which the switch from photosystem I to II in this organism was correlated with changes in the cellular nitrogen pool, and, indeed, addition of ammonium to cultures grown in either light regimen prevented phosphorylation (242). Further potential *glnB* homologs were identified by Southern blotting in several other cyanobacteria, including *Calothrix* strains PCC 7601 and PCC 7504 and *Nostoc* strain PCC 8009 (242).

A GlnB homolog was identified in *Bacillus subtilis* following the isolation of genomic fragments carrying promoters that were subject to induction under nitrogen-limiting conditions. One such promoter is that of the *nrgAB* operon, in which *nrgB* encodes a P_{II} homolog with neither Tyr-51 nor Ser-49 analogs (269). Whether the *nrgB* product is covalently modified is unknown, and its function remains to be elucidated, but comparison of the primary sequence with the structure of *E. coli* P_{II} suggests that it could possibly be phosphorylated on Ser-57 or uridylylated on Tyr-60 (36).

The recent recognition of a second *glnB*-like gene in *E. coli* raises the question whether duplicate *glnB* genes and hence duplicate P_{II} proteins are a common occurrence, and, indeed, a second copy of *glnB* has also recently been found in both *K. pneumoniae* and *Azospirillum brasilense* (53). It will be of considerable interest to determine whether any of these *glnB*-like genes are also linked to homologs of *nrgA* and to understand the roles of multiple forms of P_{II} in these organisms.

Finally, *glnB* homologs have also been identified in the methanobacteria, in which the two copies were identified between *nifH* and *nifD* (226), and even in the chloroplast of the red alga *Porphyra purpurea*, in which a *glnB*-like gene was identified through genome sequencing (205). In neither case is the role of these genes known, although in the *Methanobacterium* strains, in which only one of the genes encodes a polypeptide with a potential analog of Tyr-51, their location within a nitrogen fixation gene cluster is suggestive of a nitrogen-related regulatory role.

The ubiquity of *glnB*-like genes suggests that this component of nitrogen regulation is highly conserved in bacteria. However, it is clear that P_{II} is not always a substrate for UTase, and other primary sensors of nitrogen status are therefore likely to exist, e.g., in *Synechococcus* species. Furthermore, P_{II} could potentially serve as a signal transduction protein in other regulatory cascades, and it would be of considerable interest to examine the biology of some of these P_{II} analogs, e.g., in the archaeobacteria, in more detail.

ntrBC

ntrBC genes have been identified in *Azotobacter* (240), *Vibrio* (161), *Proteus* (230), *Thiobacillus* (126), *Rhizobium* (179, 238), *Bradyrhizobium* (166, 188), *Azorhizobium* (196), *Agrobacterium* (214, 255), *Rhodobacter* (135), and *Azospirillum* (147) species as well as in the enterics. There is no report of *ntrBC* in a gram-positive organism, although the *B. subtilis rocR* gene encodes an NtrC homolog (see below).

ntrBC genes are invariably cotranscribed, but whereas in the enterics they are part of a complex *glnA ntrBC* operon, the linkage of *glnA* and *ntrBC* has only otherwise been observed in *Vibrio alginolyticus*, *Proteus vulgaris*, and *Azotobacter vinelandii*. In *A. vinelandii*, NtrC does not regulate *glnA* expression and even though the genes are adjacent, *glnA* is not cotranscribed with *ntrBC* (241). In some cases, the transcriptional organization of the *ntrBC* genes is not known, but in at least three species, namely, *Rhizobium leguminosarum*, *Rhodobacter capsulatus*, and *Azospirillum brasilense*, the *ntrBC* operon includes a third ORF upstream of *ntrB* (79, 158, 193). This ORF is conserved in all three species and encodes a polypeptide with significant homology to the product of an ORF located upstream of the *fis* gene of *E. coli*. The role of both the *ntrBC*-

and *fis*-linked ORFs is unknown. Mutations in the *ntrBC*-linked ORF have no distinct phenotype that has yet been identified other than as a consequence of polar effects on *ntrBC* (79).

In most cases, mutational studies have been limited to inactivation of *ntrC* and the NtrC$^-$ phenotype is almost universally characterized by failure to utilize nitrate as the sole nitrogen source. Exceptions are *ntrC* mutants of *R. leguminosarum* and *R. meliloti*, which grow on 10 mM nitrate but fail to grow at lower concentrations (5, 179, 238). *R. capsulatus ntrC* (*nifR1*) mutants were initially isolated by their Nif$^-$ phenotype but are not impaired in growth on other poor nitrogen sources (135). *R. capsulatus* NtrC is only known to activate three promoters, p*nifA1*, p*nifA2*, and *glnB*p2, and in each case, transcription from these promoters is not σ^N dependent (80, 81). Hence, *R. capsulatus* NtrC represents a novel transcriptional activator that is presumed to function with an alternative and as yet unidentified sigma factor. It is of interest that *R. capsulatus* NtrC is deleted for a stretch of 18 amino acids in the central domain that are highly conserved in other σ^N-dependent activators, and this region has independently been proposed as a potential contact point between the activator and $E\sigma^N$ (181).

A second common function of NtrC is the activation of expression of *glnII* (the GSII structural gene). This is found in *R. leguminosarum* (192, 194), *Agrobacterium tumefaciens* (214), and *Bradyrhizobium japonicum* (166) and is inferred in *R. meliloti* (225). These studies refer to aerobic expression of *glnII*, and studies of microaerobic expression of *glnII* (conditions that pertain during symbiosis) in *B. japonicum* indicate that under these conditions *glnII* expression is independent of NtrC (166). One of the most striking differences between GSI and GSII in these organisms is the way in which their expression is controlled in response to nitrogen availability, with GSI levels remaining relatively constant and GSII levels being elevated substantially under conditions of nitrogen limitation. This regulation may well reflect the role of the two enzymes in nitrogen assimilation, in that GSII may be much more effective than GSI at very low levels of available fixed nitrogen. Such a role would be consistent with the occurrence of GSII in organisms whose ecology (e.g., soil microorganisms) dictates that they frequently experience severe nitrogen limitation.

In *R. meliloti*, expression of the GS encoded by *glnT* is strongly dependent on NtrC, and expression also requires *rpoN*, indicating that *glnT* expression is also under *ntr* control (49, 223).

The *ntr* genes do not appear to play a major role in symbiotic nitrogen fixation, and the symbiotic properties of *R. meliloti* and *B. japonicum* are not affected by *ntrC* mutations (166, 238). However, both *ntrC* and *ntrX* mutations in *Azorhizobium caulinodans* severely impair symbiotic nitrogen fixation, most probably via their direct effect on *nifA* expression (195). By contrast, there is evidence that nodulation (*nod*) gene expression is under nitrogen control in *R. meliloti* and *B. japonicum*, and it has been proposed that expression of the nodulation regulatory genes $nodD_3$ and *syrM* is regulated by a mechanism involving NtrC and a repressor protein, NtrR (59, 60). Such regulation would be consistent with the known effects of nitrogen-rich soils in repressing nodulation.

EVIDENCE OF Ntr-LIKE SYSTEMS IN OTHER BACTERIAL GENERA

There is some evidence for the presence of *ntr* systems in genera other than those already described. As mentioned above, *Pseudomonas putida* contains a uridylylatable P_{II}, but there are no reports of further characterization of either P_{II} or UTase or of identification of *glnB* or *glnD* genes in this organism. Genetic studies of both *P. aeruginosa* and *P. putida* have provided evidence for *ntr*-like genes. Several mutants of *P. aeruginosa* were isolated because of their inability to assimilate poor nitrogen sources such as nitrite, and a number of these were shown to have pleiotropic phenotypes with respect to nitrogen utilization (119). Pleiotropic mutants of *P. aeruginosa* which were closely linked to the *glnA* gene were also isolated, and their phenotypes could be suppressed by second-site mutations nearby, resulting in constitutive expression of many nitrogen-related enzymes including GS and urease (112, 113). This is very reminiscent of the situation in the enterics, in which mutations in *ntrB* and *ntrC* result in both of these phenotypes and are mapped close to *glnA*. An *rpoN* gene has been characterized in both *P. aeruginosa* and *P. putida*, and mutations in this gene result in a loss of ability to assimilate some forms of nitrogen such as nitrate, urea, and some amino acids; again, this mirrors the situation in the enterics (132, 239).

The apparent absence of classical nitrogen control in a number of bacterial genera may just be a reflection of the absence of sufficiently detailed studies, as evidenced by the recent recognition of some *ntr*-like genes in *Bacillus subtilis* (see below). However, while analysis of nitrogen control in other genera may yet reveal further examples of *ntr*-like systems, there is increasing evidence of alternative nitrogen regulatory pathways in prokaryotes.

NOVEL NITROGEN REGULATION SYSTEMS

Nitrogen Control in Cyanobacteria

In cyanobacteria, ammonium represses expression of the nitrate/nitrite transport system, nitrate reductase, nitrite reductase, and GSI. Expression of the GSIII structural gene, *glnN*, in *Synechocystis* strain PCC 6803 is also repressed in the presence of ammonium (209). Many cyanobacteria are capable of nitrogen fixation, and in some cases this occurs in highly developed cells termed heterocysts that differentiate from the vegetative cell filaments. Both nitrogen fixation and heterocyst development are also subject to ammonium repression.

Pleiotropic mutants of *Synechococcus* strain PCC 7942 that are unable to use inorganic nitrogen in a form other than ammonium were isolated by Vega-Palas et al. (252) and shown to have a mutation in a gene termed *ntcA*, which encodes a protein homologous to the Crp family of bacterial transcriptional regulators (251). In *Synechococcus* spp., NtcA is a positive activator of *glnA*, *nirA* (the first gene of the nitrate assimilation operon *nirA*, *nrtABCD*, *narB*) (236), and *ntcA* itself. The protein binds to target sequences in the *glnA*, *nir*, and *ntcA* promoters, which have a consensus sequence $GTAN_8TAC$ and are centered at 39.5 to 40.5 nucleotides upstream of the transcription start site (154). An identical sequence is found in the *Calothrix* strain PCC 7601 *glnA* upstream region (40, 63).

NtcA appears to be a global nitrogen regulator that plays an equivalent role in cyanobacteria to that played by NtrC in many other prokaryotes. The *ntcA* gene has been identified in 11 other cyanobacterial species, and homologous genes have been sequenced from *Synechocystis* strain PCC 6803 and *Anabaena* strain PCC 7120 (84). In *Anabaena* spp., NctA was originally identified as factor VF1, later termed BifA, which binds to sites upstream of the *xisA* gene (35, 258). Heterocyst formation in *Anabaena* spp. requires several rearrangements of the chromosome (92, 93), and one of these results in the excision of an 11-kb fragment from the coding region of the *nifD* gene by site-specific recombination of directly repeated sequences at each end of this fragment. Excision of this frag-

ment results in the formation of the complete *nifD* coding region and allows transcription of the now contiguous *nifHDK* operon. The product of the *xisA* gene, which is located in this 11-kb excised region, is required for excision of the fragment, but the precise role of NtcA in *xisA* expression is unknown (142, 258).

In *Anabaena* spp., NtcA-binding sites are present in the *glnA* and *nir* upstream regions and an *ntcA* mutant fails to induce nitrate and nitrite reductase and to express the major *glnA* transcript (RNA_I) which is induced under conditions of nitrogen limitation (83, 243). An *ntcA* mutant also fails to induce nitrogenase, but although NtcA (BifA) has been reported to bind weakly in vitro to the upstream region of the *nifH* gene (35, 203), this region does not contain sequences characteristic of NtcA-regulated promoters. Expression of nitrogenase in *Anabaena* spp. requires heterocyst development, which is in turn dependent on expression of the *hetR* gene (28). Induction of *hetR* and induction of heterocyst development are both absent in an *ntcA* mutant (83) but *hetR* promoters are also not characteristic of NtcA-dependent promoters, suggesting that a gene earlier in the developmental process than *hetR* may be the site of NtcA action.

NtcA, like some other transcriptional regulators of the Crp family, may also be able to act as a repressor, e.g., at the promoter of the *rbcLS* operon (203). RbcL encodes a subunit of ribulose-1,5-bisphosphate carboxylase which is involved in carbon fixation and therefore expressed only in vegetative cells.

To date, no sensor protein that interacts with NtcA has been identified, and although the involvement of a P_{II} protein was postulated (251), the phenotype of a *glnB* mutant in *Synechococcus* strain PCC 7942 is quite distinct from that of an *ntcA* mutant (78). If P_{II} were absolutely required for the activation of NtcA, the phenotypes should be similar, indicating that at least for the regulation of NtcA activity, P_{II} is dispensable. Thus, there may exist several branched signalling pathways originating from the primary nitrogen sensor to control nitrogen assimilation at different levels in cyanobacteria, and the primary sensor has still to be identified.

Two further nitrogen-regulated genes, *nirB* and *ntcB*, have recently been found in *Synechococcus* strain PCC 7942, in which they appear to constitute an operon (*nirB ntcB*) transcribed divergently from *nirA* (235). Transcription of *nirB ntcB* is elevated under conditions of nitrogen limitation and is NtcA dependent. The deduced protein sequence of NirB shows no similarities to known proteins, but a *nirB* mutant decreases nitrite reductase activity and excretes nitrite into the medium, suggesting that it is in some way required for maximum nitrite reductase activity. The deduced protein sequence of NtcB shows it to be a member of the LysR family of transcriptional activators, and the growth rate of an *ntcB* mutant is reduced during growth on nitrate or nitrite but not on ammonium. However, the precise role of NtcB in *Synechococcus* nitrogen metabolism is unclear.

Nitrogen Control in Gram-Positive Bacteria

There is little evidence of a classical *ntr* system in gram-positive bacteria, although some elements may exist in *Bacillus subtilis*, nor is there any suggestion of an alternative global regulatory system that characterizes this group. However, novel mechanisms of nitrogen regulation have been found in *Streptomyces coelicolor*, *Bacillus subtilis*, and *Clostridium acetobutylicum.*

Streptomyces coelicolor. Studies of nitrogen control in streptomycetes have been largely descriptive, and relatively little genetic information is available (see references 105 and 106 for reviews). A number of *Streptomyces* species have been shown to synthesize both GSI and GSII, and *glnII* genes have been cloned from *S. hygroscopicus* and *S. viridochromogenes* and identified in *S. coelicolor* by Southern blotting (17, 139). In *S. coelicolor*, GSI activity is regulated by both transcriptional and posttranslational mechanisms (75) but regulation of GSII has not been studied.

Selection of Gln^- mutants in *S. coelicolor* identified a single gene, *glnR*, which is required for *glnA* transcription and, in view of the Gln^- phenotype, is likely also to be required directly or indirectly for transcription of *glnII* (267). The *glnR* gene encodes a 29-kDa polypeptide with significant homology to other response regulator proteins in the subgroup that includes VirG and OmpR (268). These proteins form a classical N-terminal domain that includes a potentially phosphorylated aspartate residue (Asp-50 in GlnR) and a C-terminal domain which in VirG and OmpR facilitate DNA binding. Hence, GlnR may be responsible for control of *glnA* and perhaps *glnII* transcription in *S. coelicolor*, but a potential partner sensor protein has not yet been identified.

Among a set of mutants that had lost GOGAT (*glt*) activity in *S. coelicolor*, two were unable to induce histidase activity (73). The Glt^- and Hut^- phenotypes were not separable genetically even though these mutations mapped close to the other *glt* mutations, and the phenotype is reminiscent of that caused by *gltF* mutations in enteric bacteria.

A systematic analysis of nitrogen control of nitrogen catabolism enzymes in *S. clavuligerus* was undertaken, and it was shown that GS, urease, a specific protease, and arginine catabolic enzymes were subject to ammonium repression whereas enzymes involved in catabolism of serine, proline, and histidine were not (15, 16). Mutants that were deregulated for urease and GS were isolated and screened for their effects on arginine catabolism. A variety of phenotypes were found, and it was also noted that in seven of the nine mutants, GS activity had become thermolabile, a characteristic of GSII, perhaps implying that they had lost GSI activity but retained that of GSII. The genes affected in these mutants have not been characterized further, but it was noted that in each case ammonium repression of secondary metabolism as measured by cephalosporin synthesis was unaffected.

Bacillus subtilis. Studies of nitrogen control in *Bacillus subtilis* have been most detailed with respect to regulation of the GSI structural gene (*glnA*), which is part of the *glnRA* operon and is not subject to classical *ntr*-mediated regulation. Expression of *glnRA* is negatively regulated by the GlnR repressor during growth with excess nitrogen in response to an as yet unidentified metabolic signal (74, 219). GlnR is a small (135-amino-acid) dimeric protein which binds to two operators upstream of *glnRA* to inhibit transcription (97). The mechanism by which nitrogen levels is sensed is not understood, although GS appears to play an autoregulatory role. GlnR has not been shown to be modified in response to nitrogen limitation or excess, and purified GlnR binds the *glnRA* operator with high efficiency (219). Enzymes whose expression is known to be derepressed in *B. subtilis* during nitrogen-limited growth include GSI, aspartase, asparaginase, urease, and γ-aminobutyrate permease (8). However, GlnR is not known to regulate expression of any nitrogen-regulated enzyme other than GSI, indicating that another distinct nitrogen control system is present in *B. subtilis.*

Potential elements of a *ntr* system in *B. subtilis* are now emerging, e.g., the recognition of the P_{II} homolog NrgB (269). Mutations in *nrgAB* do not affect GS activity or growth on a variety of nitrogen sources including asparagine, glutamate, and urea. However, they do result in an inability to grow on

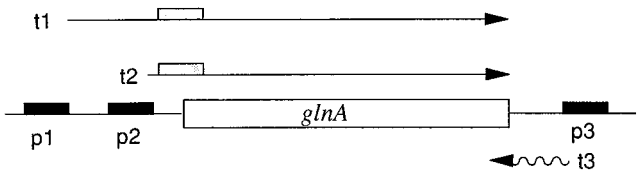

FIG. 3. Regulation of GS transcription in *Clostridium acetobutylicum*. Transcription from p1 and p2 provides mRNA transcripts (t1 and t2) coding for GS, and under conditions of excess fixed nitrogen, transcription from p3 is enhanced, resulting in the production of a short antisense mRNA (wavy line, t3), which hybridizes across the translational start sites on both of the coding transcripts (stippled boxes).

nitrate as the sole nitrogen source, which is reminiscent of *glnB* mutants in *Rhizobium leguminosarum* (5).

The *B. subtilis* σ^L protein, a homolog of σ^N, is required for growth on certain poor nitrogen sources, including arginine, ornithine, isoleucine, and valine (48), and an enhancer-binding protein, RocR, required for σ^L-dependent transcription, has been identified which is homologous to NtrC but is required only for transcription of the arginine utilization operon (29). By analogy with other two-component systems, RocR would be expected to have a partner sensor protein of the histidine kinase family, and if such a protein exists, it will be of considerable interest to determine how its activity is regulated.

Another gene that has been identified as possibly being involved in nitrogen regulation in *B. subtilis* is *outB*. Mutations in *outB* alter the ability to grow out from spores at high temperature. They also result in a reduced ability to grow with glutamine as the sole nitrogen source and the inability to derepress the *glnRA* operon (2). The sequence of the *outB* product shows 52% identity with that of the *E. coli* ammonia-dependent NAD synthetase encoded by *nadE* (2, 263), and while the mechanism of action of OutB is not known, the protein has some similarity to the receiver domains of two-component regulatory systems. It has also been shown to be phosphorylated when stationary-phase cell extracts are incubated with [γ-^{32}P]ATP (177), suggesting that this protein might respond to an environmental signal.

Clostridium acetobutylicum. GS produced by *Clostridium acetobutylicum* is reported to be regulated by the production of antisense mRNA (114). The GS gene is flanked by three promoters, two upstream, directing transcription of GS, and one downstream, producing a short transcript homologous to the start of *glnA* mRNA (Fig. 3). All three promoters are regulated by nitrogen; however, the difference in activity of each promoter directs the production of GS such that under nitrogen-limiting conditions the level of *glnA* mRNA exceeds that of the antisense mRNA by sixfold and under nitrogen-excess conditions the situation is reversed. The antisense mRNA is thought to hybridize to the sense mRNA, thereby preventing translation of nascent GS (71).

Sensing of Extracellular Nitrogen

In the classical *ntr* system, the primary nitrogen sensors are UTase and P_{II}, which respond to changes in the intracellular nitrogen pools as reflected by the ratio of glutamine to 2-ketoglutarate. There is relatively little evidence that systems also exist for monitoring the extracellular nitrogen status, but there are some indications of this in certain organisms.

In *Azorhizobium caulinodans*, *nifA* expression is regulated by *ntrBC*, but residual nitrogen-regulated *nifA* expression was found in an *ntrC* mutant (204), and a second two-component system (*ntrYX*) which shows considerable homology to *ntrBC* was subsequently identified (195). The 50-kDa *ntrX* product is significantly homologous to NtrC, and the 84-kDa *ntrY* product is homologous to NtrB, with the notable exception that it is predicted to contain two N-terminal transmembrane domains. Mutations in both *ntrC* and *ntrX* impair growth on nitrate and decrease *nifA* expression, thus influencing the symbiotic phenotype of *A. caulinodans*, which suggests that both systems contribute to nitrogen control in this organism. The putative transmembrane domains of NtrY suggest that it could be a novel nitrogen sensor involved in sensing extracellular nitrogen concentrations, and genetic experiments indicated that the two systems may well exhibit cross talk, thereby facilitating the integration of extracellular (NtrY) and intracellular (NtrB) signals.

A second potential example of an extracellular sensor is the NrgA protein of *B. subtilis*. As described previously, the *nrgAB* genes form an operon whose expression is elevated 4,000-fold in nitrogen-limited growth (8). The *nrgB* product is a member of the P_{II} protein family, and NrgA is predicted to be a 43-kDa polypeptide with a hydropathicity profile typical of that seen in membrane-bound proteins (269). It is therefore proposed that *nrgA* encodes either a transport protein or a sensor of the extracellular environment, and the transcriptional linkage to *nrgB* strongly suggests that the NrgA and NrgB proteins have a related function.

Nitrate and Nitrite Sensing

A number of examples of the specific sensing of nitrate are known in enteric bacteria, in which nitrate and nitrite serve two metabolic roles, i.e., as electron acceptors for anaerobic respiration and as nitrogen sources for biosynthesis. The respiratory nitrate and nitrite reductases and the dissimilatory nitrite reductase systems are regulated in response to nitrate and nitrite levels by twin two-component systems, NarXL and NarQP, in which the sensor proteins NarX and NarQ both respond to nitrate and nitrite (for a review, see reference 231). This is a particularly complex regulatory system, to which a further level of complexity has recently been added with the recognition that all four regulatory genes, *narXL*, *narP*, and *narQ*, are themselves regulated by nitrate (47). Synthesis of the assimilatory nitrate and nitrite reductases encoded in the *narFEDCBA* operon in *K. pneumoniae* is not only regulated by NtrC in response to nitrogen limitation but is also induced by nitrate or nitrite. This induction is controlled by the NasR protein, which is encoded immediately upstream of the *nas* operon. The mode of action of NasR and the way it senses nitrate and nitrite are unknown, although it has been noted to share some sequence similarity with the AmiR protein of *P. aeruginosa*, which is believed to function as an antiterminator (94).

Regulation of Nitrogen Fixation

A considerable body of information on nitrogen control has originated from studies of the processes that regulate bacterial nitrogen fixation in response to the availability of fixed nitrogen (for reviews, see references 72 and 169). In many cases, this regulation is mediated by classical *ntr* systems, but a number of novel forms of regulation have also been described.

In many although not all diazotrophs, expression of the nitrogen fixation (*nif*) genes is activated under nitrogen-limiting conditions by the *nif*-specific activator protein NifA, a member of the σ^N-dependent family of activators (for a review, see reference 169). Both the expression and the activity of NifA can be regulated in response to the cellular nitrogen status, and the mechanism of this regulation varies according to the organism. In *K. pneumoniae* and *Azotobacter vinelandii*,

nifA is cotranscribed with *nifL*, whose product modulates NifA activity in response to both the oxygen and the fixed nitrogen status of the cell (172, 180). NifL and NifA are produced in stoichiometric amounts, and the action of NifL on NifA appears to be by direct protein-protein interaction (12, 21, 68, 102). NifL shows some similarity to the sensor proteins of two-component regulatory systems, and in *A. vinelandii*, but not in *K. pneumoniae*, NifL contains a potential homolog of the phosphorylated histidine that characterizes the histidine protein kinases (23, 58, 202). However, alteration of this residue does not impair the regulatory properties of the protein, and there is no evidence for phosphorylation of NifL or NifA (10, 143, 265). The way in which NifL senses the cellular nitrogen status is presently unknown, but in *K. pneumoniae* it does not involve the UTase-P_{II} pathway (62, 108), suggesting either that there is an alternative nitrogen-sensing system in *K. pneumoniae* or that NifL can respond directly to the intracellular nitrogen pool. By contrast, genetic experiments with *A. vinelandii* implicate the GlnD homolog NfrX in nitrogen control by NifL, but the link between the two proteins is unclear (44).

In *Azospirillum brasilense*, no equivalent of NifL has been identified and *nifA* expression is unaffected by nitrogen status. Hence, as *nif* gene expression is repressed by fixed nitrogen, it appears that NifA activity is posttranslationally regulated (146). Mutations in both *glnA* and *glnB* allow essentially normal expression of *nifA* but severely impair expression from the NifA-dependent *nifH* promoter, suggesting that they cause NifA to remain in an inactive form regardless of nitrogen status. Furthermore, this phenotype is not suppressed by constitutive expression of plasmid-borne *nifA*. It has therefore been proposed that NifA activity is regulated directly or indirectly by P_{II} and that the Nif^- phenotype of the *glnA* mutant is caused by imbalance in the cellular nitrogen status (145). These observations suggest a novel role for P_{II} in *A. brasilense.*

Studies of *nif* expression in *Rhodobacter capsulatus*, which again does not have a NifL homolog, have also shown nitrogen repression of *nif* in strains expressing *nifA* constitutively. Attempts to exclude regulation of *rpoN* expression were unsuccessful because strains expressing *rpoN* constitutively could not be constructed. The possibility remains that the observed regulation is by posttranslational control of NifA activity by an as yet uncharacterized nitrogen control system (110), but whatever the mechanism, it does not involve NtrC, because the ammonium repression is not relieved in an *ntrC* mutant.

ADP-Ribosylation of Nitrogenase

In a number of diazotrophs, nitrogenase can be inactivated in response to transient increases in nitrogen status by the reversible ADP-ribosylation of a specific arginine residue in the Fe protein subunit of the enzyme. This involves the enzymes DraT (dinitrogenase ADP-ribosyl transferase), which adds an ADP-ribose moiety and thereby inactivates the enzyme complex, and DraG (dinitrogenase reductase activating glycohydrolase), which removes the moiety and consequently activates the enzyme (152). The addition of the ADP-ribose appears to prevent formation of the nitrogenase complex and is thought to be a completely inactivating process, allowing no fine-tuning of the nitrogenase activity similar to that which occurs with the adenylylation of GS (151). ADP-ribosylation has been demonstrated in *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, and *Rhodospirillum sphaeroides* (152), in *Azospirillum* spp. (86, 87), and in *Azotobacter chroococcum* (151), but there is no evidence for such a system in *K. pneumoniae.*

The corresponding genes for both enzymes have been cloned from *R. rubrum* and expressed in *K. pneumoniae*, and DraG and DraT were found to be both necessary and sufficient to permit the reversible regulation of nitrogenase activity in this heterologous background (85, 88). Hence, it would appear that both *R. rubrum* and *K. pneumoniae* possess a metabolic circuitry that transmits the appropriate response to DraG and/or DraT. A strain of *R. capsulatus* carrying a *glnB* mutation that results in constitutive *nif* expression still shows nitrogenase inactivation in response to ammonia shock, indicating that *R. capsulatus* has a second pathway, independent of that involving P_{II}, which is capable of communicating intracellular nitrogen status to DraT and/or DraG (98). In both *A. brasilense* and *R. rubrum*, mutations in *ntrBC* alter regulation of nitrogenase activity by NH_4^+, and at least in *A. brasilense*, this effect is apparently due to changes in the regulation of DraG activity (272, 273). The signals for the regulation of the DraG/DraT system are still unknown, but glutamine or another metabolite of NH_4^+ has been proposed, and the effect of *ntrBC* mutations could be rationalized through an effect on GS.

A Role for NAD Synthetase in Nitrogen Control?

Pleiotropic nitrogen utilization mutants have also been identified in *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*. These mutations map to a gene called *nadE* (previously called *adgA* [ammonia-dependent growth]) (3, 262). *nadE* has recently been shown to encode an ammonia-dependent NAD synthetase (263). A homologous gene to *Rhodobacter nadE* had been identified in *E. coli* and was called *efg* (essential for growth). Mutations in *Rhodobacter nadE* result in several phenotypes, reduced growth on several nitrogen sources, an inability to fix nitrogen, an inability to derepress glutamine synthetase, and a lack of the high-affinity ammonia transport system (274). The lack of Nif activity was shown to be only partially due to decreased *nif* transcription and could also reflect changes in DraG/DraT activity as a consequence of *nadE* mutation. Recent analysis of the *nadE* gene and its product has failed to indicate how mutations in this gene cause the pleiotropic effects observed; however, it may be that there is a regulatory element to the activity of NadE, as the *B. subtilis* OutB protein has been shown to be phosphorylated and, along with the *nadE* products, shares some homology with receiver domains of two-component regulatory systems (263). Alternatively, the effect of the mutations may be to prevent or reduce NAD synthesis such that nitrogen utilization is affected or to result in the increase in intracellular concentration of one of the substrates of NadE such as ammonia or NAD dinucleotide, thereby preventing the normal responses to nitrogen limitation.

CONCLUSIONS

In the last decade, our understanding of the mechanisms that underlie the regulation of cellular nitrogen metabolism in bacteria has advanced tremendously. At the molecular level, this knowledge is still largely derived from studies of the classical nitrogen regulation (*ntr*) system in enteric bacteria, but in recent years, this has begun to extend to other systems and other bacterial genera. With the recent publications of three-dimensional structures for the P_{II} protein and for the N-terminal domain of NtrC, we are approaching the position of being able to describe at the atomic level the signal transduction process that communicates the cellular nitrogen status to the transcriptional machinery. These are major advances, but there is still an enormous amount about which we know little or nothing.

The discrepancy in our knowledge of gram-negative and

gram-positive organisms is very apparent from this review, and there is clearly enormous scope for advances in this area, particularly in the major model systems. There is still much to be learned about *Bacillus subtilis*, in which phenomena such as the factors linking nitrogen starvation and sporulation (in itself a major field of study) remain largely uninvestigated. Differentiation and sporulation are also major aspects of the biology of the streptomycetes, and again nitrogen starvation is a well-recognized trigger for these events but one that is little understood at the genetic or physiological level. Furthermore, it is recognized that synthesis of many secondary metabolites in the streptomycetes is repressed by ammonium, and the implications of a detailed understanding of nitrogen control in these organisms could be of applied as well as academic interest.

The recent advances in the recognition of the role played by the NtcA protein as a global regulator in the cyanobacteria and of the alternative forms of P_{II} modification are clear examples of the tremendous advances that can be made with other bacterial groups and of the ramifications of such studies. In this case, the sensory transduction pathway has yet to be determined, and it may well bring to light new mechanisms of nitrogen sensing. Similarly, the description of a fourth family of GS enzymes is a reminder of how much we still have to learn, and with some bacteria capable of synthesizing three different forms of GS, the physiological rationale and the mechanisms that integrate the genetic control of these genes pose demanding questions.

Relatively few truly unifying themes emerge from our present state of knowledge of bacterial nitrogen control. However, the ubiquity of the P_{II} protein and its close relatives (even extending beyond the prokaryotes to the algal chloroplast) is a particularly notable feature. The crystal structure of the protein has shed new insight into the way this protein might function in signal transduction, and it would appear that in principle there is no reason why members of the GlnB family should not play a role in the transduction of signals other than nitrogen status.

Not only is the study of nitrogen control in bacteria a fascinating field of prokaryotic biology, but also it has much to offer to our understanding of cell biology in general. As is so often the case in modern cell biology, the subject has contributed in a number of areas to our recognition of the themes that can link prokaryotes and eukaryotes. The pioneering studies of the enteric *ntr* system have made a major contribution to our understanding of the function of two-component regulatory systems at the molecular level, and these studies are now informing work on the recently recognized eukaryotic two-component systems. Likewise, the recent cloning of ammonium transport genes from *Arabidopsis thaliana* and *S. cerevisiae* has offered a possible lead into the field of ammonium transport in bacteria.

We hope that this overview of one particular aspect of global regulation in bacteria will be both informative and stimulating and will encourage others to tackle some of the many problems that remain to be solved.

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